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14. ABSTRACT
The effects of battlefield injuries on the immune system are currently unknown, especially to metals such as shrapnel. Previous studies have link exposure to metal with increased immune responses (allergy). Thus battlefield injuries resulting in increased exposure to metal may sensitize individuals and lead to excessive immune responses to orthopedic implants, which many soldiers will need. The short term goal of this project is to understand whether soldiers with battle field injury and traumatic exposure to metal debris have increased immune system reactivity to metals (such as metal allergy or immune hypersensitivity alterations). We will compare the metal reactivity of immune cells isolated during a typical blood draw (6 regular blood draw tubes totaling 60mL) from soldiers exposed to metals in battle and compared with immune cell reactivity of 3 other groups of people (injured soldiers without exposure to metals fragments, non-injured healthy soldiers and non-soldiers of similar background). We expect to find that soldiers with injuries involving metal fragments will show elevated reactivity to metals and will thus be at greater risk of poor orthopedic implant outcome (e.g. Aluminum, Chromium, Cobalt Iron, Molybdenum, Nickel, Vanadium and Zirconium).

15. SUBJECT TERMS
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INTRODUCTION

PROTOCOL TITLE: Fragment Related Undetected Systemic Toxicity and Immunogenicity (Frag RUSTI)

ABSTRACT

This is a case-control study to evaluate the effect of metallic fragmentary injury sustained in the Global War on Terror on an individual's metal immune reactivity profile and systemic lead level. This study will involve 75 subjects divided into three groups of 25 subjects each: Group 1 = members who sustained a metal fragment injury, Group 2 = service members who sustained a non-penetrating injury, Group 3 = healthy civilian controls. Each group will submit a blood sample for analysis with metal-Lymphocyte Transformation Testing, cytokine release quantitative reactivity measurement, and lead level testing. The incidence and magnitude of metal reactivity and the average lead level of each group will be statistically compared.

OBJECTIVES/SPECIFIC AIMS/RESEARCH QUESTIONS.

The purpose of this study is to evaluate the effect of retained metallic fragments sustained from IED, mortar, artillery, and grenade attacks on a patient's immune system reactivity to metals.

Primary objective: To compare subjects with a history of metal fragment injury versus control subjects by determining the incidence and magnitude of metal reactivity with metal-lymphocyte transformation testing.

Secondary Objective: To compare subjects with a history of metal fragment injury versus control subjects by evaluating serum lead level

should be removed9, 13. This study is specifically interested in determining whether U.S. soldiers with residual metallic fragments demonstrate elevated serum lead levels.

1. SUMMARY: The effects of battlefield injuries on the immune system are currently unknown, particularly those that involve exposure to metal, e.g. shrapnel. We have previously linked increased exposure to metal with increased incidence of metal reactivity. This, together with past reports of metal reactivity associated with decreased implant performance, suggests that battlefield injuries resulting in increased exposure to metal will sensitize the individual and lead to excessive immune responses to orthopedic implants, thus compromising their long term performance. The short term goal of this project is to understand whether soldiers with battle field injury and traumatic exposure to metal debris have increased immune reactivity (adaptive immune responses) to metals and thus establish if excessive immune responses to implant debris may affect long term orthopedic implant performance.

2. BACKGROUND AND SIGNIFICANCE: While injury to soldiers on the battlefield is increasingly coming from improvised explosive devices (IEDs), improved protection from body armor, ballistic helmets, and vehicle armor are improving the wound injury. This has lead to more soldiers surviving major blasts, where an increasing amount of injury is limited to the extremities. Improved survival has allowed doctors to focus on limb salvage and restoring normal function.[1] The metal fragments that remain in situ either temporarily (capable of being removed) or fragments incapable of removal due to practical or technical limitations are a continuing source of metal exposure in vivo.[1] For those that require orthopedic implants this traumatic exposure to metal debris and the continuing source of metal release is likely to impact their immune system. The central question is whether this metal debris affects both the short and long term immune system metal reactivity?

Biologic reactivity to orthopedic implant debris represents a less severe version of metal fragment exposure, and is a phenomenon that has been well studied. While the primary goal of the immune system is to protect against harmful pathogens, it also leads unwanted reactivity to implant debris. Both pathogen protection and unwanted reactivity to implant debris are controlled by the sequential activation of innate and adaptive immune systems. Generally, the adaptive immune system involves the production of a very large repertoire of antigen-specific cells. An innate immune response is generally less specific but quicker and provides essential signals for adaptive system activation [2]. General progressive inflammatory reactivity to implant debris is attributed to innate immune responses, (i.e. macrophage induced inflammation) [3-11]. Excessive reactivity to implant debris is well documented in case and group studies, however, it remains a relatively unpredictable and poorly understood phenomenon [12-14]. There are reports

of exuberant dermal and inflammatory responses temporally associated with the implantation of metal implant components [13,15-26]. Metal ions complexed with protein are considered to be candidate antigens (or more loosely termed, allergens) for eliciting adaptive immune responses. Metal sensitizers include beryllium,[27] nickel,[27-30] cobalt[27] and chromium,[27] and occasionally tantalum,[31] titanium[32,33] and vanadium [31]. Nickel is the most common metal sensitizer (with 10-15% of the general population "nickel allergic") followed by cobalt and chromium [12,28-30].

Retained Metal Fragments: In common practice, the metallic fragments sustained by gunshot, IED, or grenade injury are left to remain inert in soft tissues [34,35]. Retained foreign bodies are indicated for subacute removal when they are located near or within a joint, in weight bearing areas, or in proximity to neurovascular structures. Fragments located in extra-articular or extra-thecal soft tissue are believed to be encased in fibrous tissue and are considered inert Peyser, 2006 10437 /id}. Routine removal has not been recommended due to the possible morbidity accompanying surgery. Reports do exist, however, of lead foreign bodies in soft tissue causing plumbism [36-38]. There are no strict guidelines as to when patients with gunshot or other metal fragment wounds should be tested to determine serum lead concentration. Previous studies revealed that blood lead concentration in patients with extra-articular gunshot wounds was below hazardous limits as determined by the Occupational Safety and Health Administration [37 39]. To the best of our knowledge, no research exists to date on lead levels resulting from metal fragment injury sustained in the Global War on Terror.

2.1 Hypothesis: This study will test the hypothesis that the retained metals like those found in mortar, artillery, and grenade fragments contribute to impaired or hyper immune system reactivity to metals.

2.2 Objective: The goal of this study is to test this hypothesis in patient cohorts of soldiers recuperating from battle field injury with retained metal fragments and compare the immune metal-reactivity of these individuals to injured and non-injured soldiers without metal fragment exposure, to determine if changes in immune system reactivity to metals have occurred.

This reactivity is important to orthopedic implant survival in the following manner. All metals in contact with biological systems corrode[40,41] and the released ions activate the immune system[42-44]. "Metal allergy" or metal reactivity to particulate and soluble (non-particulate) metal debris is clinically characterized by symptoms of an overly aggressive immune response and is increasingly being implicated as a failure mechanism in patients with metal-on-metal total hip replacements and surface replacement arthroplasty of the hip [45-47]. Orthopedic implant metals known to be sensitizers (haptenic moieties in antigens) include nickel,[27-30] cobalt[27] and chromium,[27] while occasional responses have been reported to tantalum,[31] titanium[32,33] and vanadium[31].

Patients with total joint replacements are all exposed to billions of metal, polymer, and/or ceramic particles generated by implant degradation (wear and corrosion) in vivo, which accumulate adjacent to the implant and bone, and induce inflammatory cell/tissue reactions. Bone loss around implants does not occur to the same extent in patients with similar rates of implant wear. It is commonly noted that some individuals with severely worn components can demonstrate little periprosthetic bone loss, while others with modest amounts of wear can demonstrate extensive osteolysis and implant loosening. Traumatic injury and exposure to metal fragment is likely to affect this inherent "state" of metal-debris-reactivity.

Recent reports indicate the presence of lymphocytic involvement in aseptic osteolytic lesions around failing metal-on-metal articulating implants, where there are abnormally high levels of metal [48,49]. Cell mediated delayed type hypersensitivity is characterized by antigen activation of sensitized T-helper lymphocytes releasing various cytokines which result in the recruitment and activation of macrophages. These subset populations of T helper (Th) lymphocytes involved in metal implant responses are purported to be of the CD4+ Th1 subtype. This Th1 subpopulation of T cells is characterized by their cytokine release profile, e.g. interferon-gamma (IFN-g tumor necrosis factor-alpha (TNF-a), interleukin-1 (IL-1) and interleukin-2 (IL-2). These inflammatory cytokines associated with Th1 lymphocyte reactivity is important to the pathogenesis of aseptic osteolysis.[50,51] The extent to which soldiers exposed to metal fragments have an altered immune response to metals and thus a heightened immune response to implant debris leading to early implant failure, has not been assessed in any way, and remains an unanswered question.

2.3 RELEVANCE:

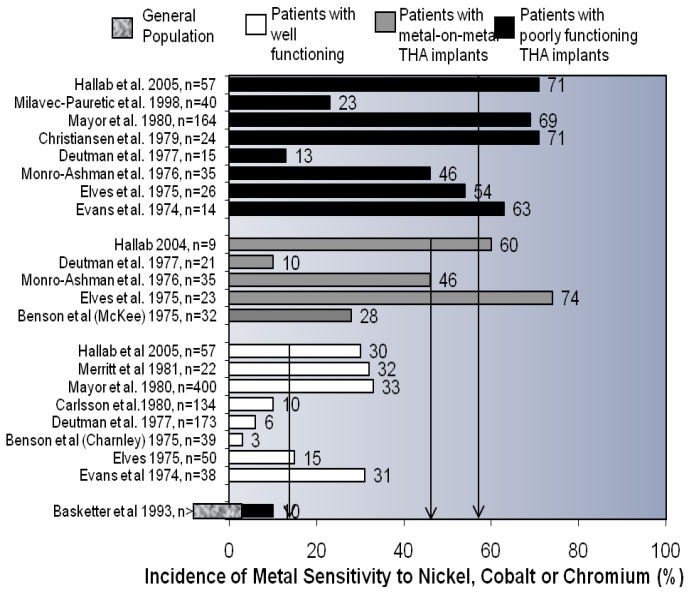
The Global War on Terrorism (GWOT) represents the longest ongoing engagement in United States history. U.S. Military operations in Iraq and Afghanistan have resulted in over 57,000 casualties to date1. Unconventional warfare, such as improvised explosive devices, roadside bombs, and rocket-propelled grenades have led to more severe injury patterns. The concurrent development of more advanced protective equipment and the institution of a rapid medical evacuation system have allowed many servicemembers to survive these serious, previously lethal injuries.

Musculoskeletal injures are the most common battlefield wounds sustained in modern combat, accounting for 54% of all combat wounds and requiring upwards of 60% of healthcare resources2. Cross and colleagues, in a review of U.S Army Physical Evaluation Board records of combat-injured soldiers, report that 69% of long-term disabling conditions are orthopaedic in nature3. Degenerative arthritis was the most common unfitting condition. Given this data, it is highly likely that many GWOT combat injured will one day require treatment of post-traumatic arthritis, to include possible hip or knee arthroplasty.

Metal reactivity, or metal "allergy", is increasingly being implicated as a failure mechanism in certain types of hip arthroplasty prostheses [45,52,53]. In hip implant patients, elevated metal ion levels have been positively correlated with lymphocyte reactivity and implant failure [49,54]. This study will evaluate the extent to which U.S. soldiers exposed to metal fragments have an altered immune response to metals, and consequently a higher risk of future arthoplasty implant failure.

Studies characterizing the nature of combat and extremity wounds in the Global War on Terror have established that gunshot wounds and explosions cause >95% of battle injuries [34,55]. The penetrating injures resulting from improvised explosive, mortar, and grenade blasts are inflicted by high-velocity metal fragments energized by the explosion. These fragments are made up of various metal alloys, including lead. They most often affect the musculoskeletal system, where fragments can cause local anatomic damage and possible resultant infection9. Many of these metal fragments remain in the affected servicemember's soft or osseous tissues until they cause local irritation or systemic toxicity. Lead-containing metallic fragments can cause plumbism [36,37,56]. There is an ongoing debate in the orthopaedic literature as to when and why these residual metallic foreign bodies

3. PRELIMINARY RESULTS: Studies have shown that people with orthopedic implants have a higher incidence of metal reactivity (20%) than the general population (10%), (**Fig. 1**). Furthermore people with failing implants have even higher incidence of metal reactivity (approx 60%) comparable to people with metal-on-metal implants with which have an incidence of metal reactivity of approx 50%. We have reported that people with the highest serum metal ion concentrations (Co and Cr) had metal-on-metal bearing surfaces. In the past these elevated metal ion concentrations demonstrated a positive correlation with lymphocyte reactivity, indicating a link between metal exposure and orthopedic implant performance [45-47].



4. RESEARCH PLAN: If metal fragment exposure during trauma is enough of a stimulus to significantly change immune system reactivity, then soldiers exposed to this in battle will demonstrate altered metal-reactivity profiles when compared to injured soldiers without exposure to metals fragments and age/gender matched soldiers and non-combatants of similar background that have not been exposed to injury or metal debris.

4.1 Subject Groups: We will compare metal immune (T-cell) reactivity profiles of 4 different groups of soldiers, using metal-Lymphocyte Transformation Testing (metal-LTT) assays, flow cytometry and cytokine analysis (Table 1, Groups: 1) soldiers with metal-fragment injury (3-6 months post-injury, 2) soldiers with non-metal fragment injury, and 3) non-soldier matched controls (n=25 in each group). Subject involvement is limited to a 60mL blood draw, which will be sent to the PI's institution for analysis. All subjects will be recruited by self referral via flyers put up at medical centers that treat wounded soldiers. The consent process is described in the following paragraph. All blood draws will be the responsibility of the subject once they receive the kit, and as stated in the consent form will have to have their blood drawn at their local VA, Rush University Medical Center, your primary care physician or a local qualified phlebotomist. .

Table 1. Number of subjects in Groups 3a-3d for lymphocyte and monocyte responses at a single time point (6month-5 years post-operative).

Subgroups	Subjects in Group	Subject Recruited from
Group 1 Soldiers w/ metal-fragment injury	25	Brooke Army Medical Center
Group 2 Soldiers w/ non-metal injury	25	Brooke Army Medical Center
Group 3 Controls (healthy non-soldiers)	25	Rush University Medical Center

4.2 Inclusion and Exclusion Criteria.

Inclusion Criteria:

Group 1: Soldiers at least 3-6 months status-post metal fragmentary injury (recruited at Brooke Army Medical Center)

Group 2: Soldiers status post blunt mechanism injury (recruited at Brooke Army Medical Center)

Group 3: Healthy non-soldiers (recruited at Rush University Medical Center)

Exclusion Criteria:

Inability to complete health questionnaire or undergo blood draw (all groups)

Group 2: Blast injury more recent than 3 months time.

4.3 Subject Screening Procedures. Subjects will be screened regarding inclusion and exclusion criteria. No physical examination will be conducted either before or after recruitment. Informed consent will be obtained prior to completion of the study questionnaire.

4.4 Description of the Recruitment Process. Subjects will be recruited by flyers and advertisements posted at Brooke Army Medical Center or at Rush University Medical Center. Patients eligible for the study will be offered participation during Orthopaedic and Rehabilitation department clinic visits. No physical examination will occur as part of this study. Patients will be deemed eligible for the study based on their screening for inclusion or exclusion by the clinical nurse research coordinator.

4.5 Consent Process. The study will be explained by a clinical nurse research coordinator included in the study personnel or alternatively by one of the investigators. Consent will be obtained by the investigator or research coordinator, who is trained in consenting procedure. Consent will occur prior to completion of the study questionnaire and blood sample. At the subject's request, time for decision making may be allowed. The subject may elect to undergo blood draw immediately following consent or at a later date.

4.6. Study Procedures/Research Interventions: Patients will self-recruit by responding to study advertisements, or will be offered participation in the study during Department of Orthopaedics and Rehabilitation clinical visits, should they fit inclusion criteria. The patient will be initially screened for inclusion/exclusion by the clinical research nurse.

Following study inclusion, the subject will meet with the clinical nurse research coordinator. The study will be reviewed in detail and informed consent will be obtained. After informed consent, the patient will be assigned a subject ID and the process of obtaining study data and the study blood specimen will proceed. The patient may elect either to complete all study participation that day, or may choose to return at a later time following the informed consent.

All subjects will complete the study questionnaire, directing any questions to the research nurse. A single 60mL blood sample will then be obtained by venipuncture by a clinician experienced in

phlebotomy. The blood sample will be sent immediately to Rush University Medical Center for metal reactivity analysis. Should the sample be unacceptable for analysis, the clinical nurse research coordinator will contact the patient, who will be presented with the option of giving a repeat blood sample or voluntary withdrawal from the study.

At the time of blood draw, the subject may elect to receive their metal reactivity and lead level results when available. Should the subject choose this option, the results of testing will be provided to the patient in person or by conventional mail in approximately 4 weeks time.

4.7 Compensation for participation. Subjects will be compensated upon enrollment in the study. Subjects will be compensated upon enrollment and completion of the informed consent, health questionnaire, and blood sample. Subjects will be compensated with a $50.00 VISA check card.

4.8 Laboratory evaluations and special precautions. Blood samples will be collected in sodium heparinized tubes (standard green topped vacutainers). These are the standard used by CLIA approved human diagnostic laboratories around the United States for overnight shipment of blood for use in highly complex immunology testing such as Lymphocyte Proliferation Testing and Flow Cytometry analysis of activation markers and PBMC populations. The chances are less than 1% of the necessity of a redraw when blood is received by the lab within 24 hours. All blood specimens will be sent to Rush via priority overnight shipping in appropriate mailing containers supplied by Rush. Mailed specimens will be labeled by subject ID and will not contain any patient identifying information. Upon arrival at Rush, metal-Lymphocyte Transformation Testing, flow cytometry, cytokine testing, and lead level testing will be performed. Should the blood sample need to be repeated, the patient will be contacted by the clinical nurse research coordinator and may either elect to undergo repeat venipuncture or voluntarily withdraw from the study.

4.9 Specimen storage. All the blood received from each sample will be immediately used/processed/analyzed and none of the original samples will be stored for future use.

4.10 Data Collection. Subjects will be assigned a subject identification number, to consist of their group number and a second number indicating their individual number within the group. For example: Subject # 20 in group 1 will be assigned subject ID 1-20. A study questionnaire regarding the nature of the patient's injury and relevant past medical history will be obtained. Please see the study questionnaire submitted with this protocol. No personal information (e.g. legal status or participation in illegal activities) will be collected from the subjects. All collected information will be limited to that related to injury status and history of immune status, that are part of the study questionnaire.

Laboratory data to be collected includes a Metal-LTT analysis panel, blood flow cytometry, cytokine analysis, and serum lead level.

4.11 Human Biological Specimens/Tissue/Data Banking. All specimens will undergo one time analysis. No sample will be stored for future use.

4.12 Statistical Consideration

Sample Size Estimation. We anticipate that a sample size of 25 in each group will have an >80% power to detect a probability of 0.73 that an observation in one group is less than an observation in the other group using a two-sided Mann-Whitney test with a 0.05 significance level. The power calculations were obtained using Query Advisor (version 2).

Estimate Required Sample Size	**75**
Estimate Participant Drop Out	**0**
Estimate Participant Withdrawal	**5**
Total Enrollment Requirement	**80**

Enrollment at Each Site	
BAMC	**50**
Rush University Medical Center	**25**

4.13 Primary (i.e., primary outcome variables) and secondary endpoints.

Primary objective: To compare subjects with a history of metal fragment injury versus control subjects by determining the incidence and magnitude of metal reactivity with metal-lymphocyte transformation testing.

Secondary Objective: To compare subjects with a history of metal fragment injury versus control subjects by evaluating serum lead level

4.14 Data analysis.

Metal Reactivity: Metal-lymphocyte transformation testing will be performed to quantify metal reactivity on a scale of lymphocyte stimulation index. The incidence of metal reactivity, defined by a simulation index>2, of groups 1-3 will be compared. The average lymphocyte reactivity to each metal of subjects in each group will be compared. Sub-group analysis will include determination of reactivity as a function of the amount of time elapsed since metal fragment injury in group 1. Reactivity between groups will be analyzed using a Student's t-test for independent variables or a Mann-Whitney test. Should data not be normally distributed, a Kruskall-Wallis non-parametric analysis of variance will be employed. Factors such as chronic medications, co-morbidities, presence of orthopaedic implants, age, and other environmental conditions (such as occupational exposure to metal debris) may confound the interpretation of systemic lymphocyte reactivity and will be controlled as much as possible.

Serum Lead Level: Serum lead concentration will be obtained. Average serum lead level of subjects in each group will be compared using the previously mentioned statistical methods. Sub-group analyses within group 2 will compare serum lead level of subjects as a function of the amount of time elapsed since metal fragment injury and the type of metal fragment injury (IED, gunshot, grenade).

4.15 Confidentiality.

Hard and electronic copies of questionnaire information will be retained for the duration of the study. Hard copy data will be stored in a locked file cabinet within a locked room in the department of orthopaedics and rehabilitation. Electronic data will be stored on a CAC-protected computer located within a locked room in the department of orthopaedics and rehabilitation.

Blood testing results will be stored electronically in a password protected computer. The results of metal reactivity testing will not be sent to subject unless requested by the subject. The results of lead level testing will not be sent to subject unless requested by the subject. Should the patient demonstrate lead levels above hazardous limit, they will be notified of their result as soon as possible telephonically or in person.

4.16 Certificate of Confidentiality. N/A

4.17 RISKS/BENEFITS ASSESSMENT

4.17.1 Risks. Physical risks to the subject are the complications of venipuncture, to include minor bruising/hematoma (12.3%), diaphoresis with hypotension (2.6%), syncope (<1%), and cellulitis or phlebitis (<1%). Standard aseptic procedure will be employed during sample collection and blood draws will be conducted by a clinician experienced in phlebotomy.

4.17.2 Potential Benefits. Potential benefits to the subject include knowledge of their metal reactivity profile and lead level.

4.18 ADVERSE EVENTS, UNANTICIPATED PROBLEMS, AND DEVIATIONS

Possible adverse events

Possible adverse events following phlebotomy include:

- Minor bruising/hematoma (12.3%)
- Diaphoresis with hypotension (2.6%)
- Syncope (<1%)
- Cellulitis or Phlebitis (<1%)

The following information will be collected for all AEs:

- Date/time of onset
- Description of the AE
- Severity
- Relationship to phlebotomy

- Action taken
- Outcome
- Date of resolution

If treatment was required for the AE, this will be recorded including the type of treatment, duration and any other relevant details. Adverse event information will be captured on source documents. The investigator will sign off on each individual adverse event.

4.19 Reporting Unanticipated Problems Involving Risks to Subjects or Others, Serious Adverse Events and Deaths to the Office of the IRB, RUMC and BAMC.

Unanticipated Problems: An unanticipated problem is an unforeseen event that occurs during the course of a research trial that potentially increases the risk to participants or others; adversely affects the rights, safety, or welfare of participants; or affects the integrity of the study. All unanticipated problems involving risks to human subjects or others will be reported promptly to the IRB.

Monitoring for Serious Adverse Events A serious adverse event (SAE) is defined as any untoward medical occurrence that: results in death, is life – threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in a persistent or significant disability/incapacity, or is an important medical event.

Timing: Any SAE occurring in a subject after providing informed consent until 30 days after completing the study will be recorded and reported.

Reporting: All unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths will be reported within three (3) business days by phone (210-916-0607), by e-mail (BAMC_IRB_AE@amedd.army.mil), by facsimile (210-916-1650) or via letter addressed to Human Protections Administrator, Office of the Institutional Review Board, Department of Clinical Investigation, Brooke Army Medical Center, 3698 Chambers Pass, Fort Sam Houston, TX 78234-6315. A complete written report will follow the initial notification.

4.20 WITHDRAWAL FROM STUDY PARTICIPATION.

Subjects may withdraw from participation in the study at any time during the study for any reason. Should the subject decide to withdraw from the study, the patient's name will be removed from study records. His or her study questionnaire will be destroyed. The subject's blood sample will be discarded if it is pending analysis. There are no negative consequences of withdrawal from this study. The subject's participation may be terminated if the investigator or clinical nurse research coordinator perceives any risk to the subject's safety.

4.21 Privacy/Sensitive Information: No sensitive information will be collected other than health related information that may impact the interpretation of immune responses to metals. No personal information (e.g. legal status or participation in illegal activities) will be collected from the subjects or their primary care doctors. All collected information will be limited to that related to injury status and history of immune status, that are part of the study questionnaire (see **Appendix B**). The results research testing will not be sent to the subjects' doctors unless requested by the subject. In addition the results of all tests and provided medical history will be coded and locked in the PIs office and computer and the key to the code will kept in a separate locked file. Every effort will be made to keep participation and all results strictly confidential. Results of the testing will be shared only with the subject and by law with the Government if they request so. Results will be sent to the subject's physicians if the subject participant requests it.

4.22 Key Personnel: At Rush University Medical Center: There will be four key personnel all from Rush University Medical Center: Dr Nadim Hallab, Dr Joshua Jacobs, and Kyron McAlllister. Only Dr Hallab, Dr Jacobs and Mr McAllister will have access to subjects information. Nadim Hallab., Ph.D. - Principal Investigator will be responsible for the overall coordination and execution of the project. He will provide intellectual and technical expertise, collect and process data and prepare reports and manuscripts for publication. Dr Joseph L. Petfield, MD CPT, USA MC, Department of Orthopaedics and Rehabilitation San Antonio Military Medical Center, a co-Investigator will be responsible/direct all aspects of the study to be conducted at Brooke Army Medical Center and will have access to all subject information. Additionally Dr. Joseph Hsu MD, a prior US Army orthopaedic traumatologist,) is a co-Investigator that will help direct and interpret data obtained from testing.

4.23 Anticipated Results We anticipate that soldiers with injuries involving metal fragments will show elevated reactivity to metals (e.g. Aluminum, Chromium, Cobalt Iron, Molybdenum, Nickel, Vanadium and Zirconium) and thus will be at risk of poor orthopedic implant outcome. Knowledge of this condition is vital to judicious implant selection and post-operative management for soldiers that require indwelling orthopedic implants.

4.24 Potential limitations, difficulties and alternative approaches. Our laboratory has a long history of LTT with metal antigens that are proxies of implant debris, thus we do not expect methodological problems associated with recruiting and testing for "metal-allergy" (DTH-like) response in cohorts. Positive T-cell metal reactivity (defined here and in past studies [57-60] as an SI>2 to a metal challenge agent) may imply but does not prove a state of in vivo excessive reactivity to metal implant debris or so called metal sensitivity. 1) We anticipate that a sample size of 25 in each group will have an >80% power to detect a probability of 0.73 that an observation in one group is less than an observation in the other group using a two-sided Mann-Whitney test with a 0.05 significance level. The power calculations were obtained using Query Advisor (version 2). Factors such as chronic medications, co-morbidities, the presence of multiple implants, age, and other environmental conditions (such as occupational exposure to metal debris) may confound the interpretation of systemic lymphocyte reactivity and will be controlled as much as possible.

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BODY: ***Current Status:***

The Study is finalizing the recruitment procedures for human soldier subjects because final approval for subject recruitment has not been obtained from the DOD. Procedual difficulties have resulted from 1) the inability to partner with an appropriate Army medical doctor co-investigator at medical facilities such as WRAMC for reasons detailed in th following sections and, 2) the eventual changing of the the study protocol to adapt to this situation such that soldiers can self recruit where the entire study is now conducted at Rush University Medical Center.

Original protocols, consent forms etc were approved by the Rush University Medical Center IRB. Subsequently they needed amending to change the recruitment site to exclusively that of the PI, Rush University Medical Center. These amended consents and and protocols were then reviewed by the Human Research Protection Office at the U.S. Army Medical Research & Materiel Command and a list of changes were requested. These requested changes were then made and the amended protocols and consents were again processed and preliminarily approved by the Human Research Protection Office at the U.S. Army Medical Research & Materiel Command, pending Rush IRB approval. These provisionally approved amendents to the original approved protocols and consents and been re-approved by the PIs institutional review board (Rush University Medical Center) and these amended protocols have been sent to the Human Research Protection Office at the U.S. Army Medical Research & Materiel Command for final approval prior to the putting up of fliers and beginning the recrutement of subjects.

Past Year Effort:

The following details our good faith efforts to attempts to conduct the study on time while trying to accommodate the changing ground conditions and requirements.

1) We have been successful in recruiting an army collaborator: Dr Joseph L. Petfield, MD CPT, USA MC, Department of Orthopaedics and Rehabilitation San Antonio Military Medical Center
2) The protocol and consent forms were changed considerabley from the original approved process. These changes are summarized below.

Amendment needed to accommodate requirements of IRB at Brook Army Medical Center in San Antonio for consistency of approved Rush and BAMC protocols. There has been no change to risk profile (participation is still limited to a one time blood draw and short questionnaire) and additional lab testing has been added (e.g. Lead level testing) thus improving benefits to study participants. There have been no substantive changes to the procedures, analysis or study subjects.

Specific areas amended

1) Title change
2) Addition of Dr Joseph Petfeild and Dr Joseph Hsu (Army Orthopedic Surgeons) to the study.
3) Reduction of number of groups and study subjects.
4) Addition of extra metal ion assays for Lead.
5) More specific inclusion critera, i.e. “Soldiers at least 3-6 months status-post metal fragmentary injury (recruited at Brooke Army Medical Center)”.
6) Addition of $50 compensation to study participants.
7) Great written detail in subject recruitment, testing and data storage and safety.

KEY RESEARCH ACCOMPLISHMENTS: Pending.

We have over the past year determined means to start recruitment without a dedicated Army physician.

REPORTABLE OUTCOMES: Pending start of recruitment of subjects.

CONCLUSION: The Study is currently waiting for final approval from the DOD to begin recruitment at Department of Orthopaedics and Rehabilitation San Antonio Military Medical Center.

This delay has been due to unavoidable procedural difficulties have resulted from 1) the inability to partner with an appropriate Army medical doctor co-investigator at medical facilities such as WRAMC for reasons detailed in th following sections and, 2) the eventual changing of the study protocol to adapt to this situation such that soldiers can self recruit where the entire study is now conducted at Rush University Medical Center. Scientific finding are pending.

REFERENCES: None

APPENDICES:

SUPPORTING DATA: Pending final data.